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ORIGINAL ARTICLE

Effects of Dietary Vitamins A, B_2, and B_6 Supplementation on Growth and Feed Utilization of Juvenile Chinese Soft-shelled Turtle *Pelodiscus sinensis* according to an Orthogonal Array Experiment

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Abstract An orthogonal experimental design OA_9 (3^3) was used to evaluate the effects of vitamins (A, B_2, and B_6) on the growth and digestive ability of the juvenile Chinese soft-shelled turtle, *Pelodiscus sinensis* (initial weight, 5.9±0.2 g). A total of 135 turtles were divided into 9 groups, which each included 15 individuals. The results revealed that vitamin A (VA) had the strongest impacts on the growth rate and feed utilization among the three vitamins; 35,000 IU kg^{-1} VA had optimal effects on the feeding intake and specific growth rate, and 20,000 IU kg^{-1} VA had optimal effects on protein digestibility and the feed conversion ratio. Vitamin B_2 (VB_2) was essential for regulating protein deposition and the energy efficiency for growth of the turtles; 120 mg kg^{-1} VB_2 resulted in increased protein and energy deposition, and 180 mg kg^{-1} VB_2 had greater beneficial effects on the growth rate. Vitamin B_6 (VB_6) had important effects on protein and feed efficiency; however, VB_6 at an excessive level (120 mg kg^{-1}) restricted turtle growth. Based on the above growth results, dietary supplementation of VA, VB_2 and VB_6 at levels of 35,000 IU kg^{-1}, 180 mg kg^{-1} and 70 mg kg^{-1}, respectively, were recommended for the juvenile soft-shelled turtle.

Keywords *Pelodiscus sinensis*, vitamin, growth performance, digestion capacity, orthogonal design

1. Introduction

The Chinese soft-shelled turtle, *Pelodiscus sinensis,* is one of the most commercially important reptile species in China (Xie *et al*., 2012; Pu and Niu, 2013), and its total production reached 341,288 tons in 2014 (Fisheries Department of Agriculture Ministry of China, 2014). The researches on the bioenergetics and nutritional requirements of soft turtles have been reported (Nuangsaeng and Boonyaratapalin, 2001; Huang *et al*., 2003; Huang and Lin, 2004; Zhou *et al*., 2004; Hou *et al*., 2013; Chen and Huang, 2014); however, supplementation of the diets of these reptiles with several vitamins must be optimized for better growth performance. Vitamins play many important roles in the growth, physiology and metabolism of developing animals (Halver, 2003) and can affect the feeding and skeletal development of larval fish (Fernández and Gisbert, 2011; Reham *et al*., 2013). The availability of vitamins at optimal levels is essential for normal animal growth. Previous studies have shown that vitamin A (VA) (Yutaka *et al*., 2011; Chen and Huang, 2014), B_2 (VB_2) (Deng and Wilson, 2003) and B_6 (VB_6) (Giri *et al*., 1997) are essential for animal growth.

Among these vitamins, VA (retinoids) includes a group of compounds that are structurally similar and exhibit biological activity due to retinol; these compounds bind to or activate a specific receptor or group of receptors (Hemre *et al*., 2004; Reham *et al*., 2013). VA is essential for maintenance of normal vision and growth in fish (Olson, 1991; Funkenstein, 2001); in addition, it enhances development of the alimentary tract (Lahov and Regelson, 1996). Previous studies have shown that

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all vertebrate species can suffer from VA deficiency and/or toxicity, and the biological consequences of both deficiency and toxicity are similar among most species. Normal growth and reproduction can only be sustained in the presence of optimal VA levels (Stéphanie *et al*., 2010). The requirement for VA in turtles has been shown to be approximately 2.5–3.5 mg kg^{-1} in a single factor experiment (Chen and Huang, 2014).

Riboflavin (VB_2) is a water-soluble vitamin required by all animals (Deng and Wilson, 2003; Souto *et al*., 2008). It cannot be synthesized by monogastric animals, which must therefore consume foods with sufficient VB_2 levels to meet their metabolic demands (Kavita *et al*., 1996). A low VB_2 level, especially in fish, results in several signs of gross deficiency, including high mortality, uncoordinated swimming, photophobia, cataracts, dark skin coloration, low feed conversion efficiency, cornea and eye lens opacity, and dark body pigmentation (NRC, 1993; Deng and Wilson, 2003); in addition, high dietary VB_2 intake is necessary to support maximum weight gain in fish (Serrini *et al*., 1996).

VB_6 is the precursor of the coenzyme pyridoxal phosphate, which is required for the non-oxidative degradation of amino acids through transamination, deamination, and desulfuration. VB_6 metabolism is related to dietary protein or amino acid metabolism in animals (Hilton, 1989; Giri *et al*., 1997), and the structures and functions of digestive and immune system in fish are affected by this vitamin (He *et al*., 2009; Feng *et al*., 2010; Li *et al*., 2010). Due to its multiple roles in various metabolic processes, a number of potential signs are indicative a VB_6 deficiency in animals. In fish, these signs include anorexia, anemia, dark coloration, loss of equilibrium, poor growth, and high mortality (Albrektsen *et al*., 1993; Giri *et al*., 1997). However, little information is available on the dietary VB_2 and VB_6 requirement of the soft-shelled turtle.

Many experiments have been conducted investigating VA, VB_2 and VB_6 requirements in aquatic animals (Halver, 1989; Serrini *et al*., 1996; Shiau and Chen, 2000; Lin *et al*., 2003; Stéphanie *et al*., 2010), and most studies on vitamin requirements have examined a single vitamin. However, assessments of vitamin combinations may yield a more realistic representation of vitamin requirements in animals, as appropriate combinations of VB_2, VB_6, niacin and pantothenic acid have been shown to improve the growth and meat quality of crucian carps (Lin *et al*., 2003). Tan *et al*. (2007) used an orthogonal design to evaluate the possible nutritional functions of vitamins A, D_3, E, and C on gonadal development and the immune response of yearling eel. An orthogonal array design is a useful statistical tool for multi-factor analyses that can reflect a general condition with the fewest number of experimental trials and can be used to determine dominant contributing factors, as well as the appropriate combination of levels of each factor (Montgomery, 1991; Zheng and Jiang, 2003). Few experiments have been conducted to determine the vitamin requirements of fish according to an orthogonal design (Rong *et al*., 1996; Lin *et al*., 2003). In previous studies, the recommended dietary VA, VB_2 and VB_6 requirements for the soft-shelled turtle were determined according to production experience, but limited information is available about the effects of these 3 vitamins on the growth of this reptile species.

The present study was conducted to explore the effects of vitamins on feeding, growth and protein utilization of juvenile soft-shelled turtles using an orthogonal experimental design. The findings may aid in providing a basis to further optimize the vitamin supplementation in turtles' diets.

2. Materials and Methods

2.1 Experimental design The study was performed in a laboratory at Hebei Normal University, Shijiazhuang, Hebei Province, China. We used an $OA_9 3^3$ experimental design to study the effects of dietary supplementation of 3 vitamins at 3 levels (VA: 5000, 20,000 and 35,000 IU kg^{-1}; VB_2: 60, 120 and 180 mg kg^{-1}; and VB_6: 20, 70 and 120 mg kg^{-1}) on the growth and development of soft-shelled turtles (Table 1). An orthogonal array design was used to determine which vitamin had the strongest effects on feeding, growth and protein utilization efficiency of soft-shelled turtles. In this experiment, 135 turtles were divided into 9 groups, which each included 15 individuals.

2.2 Experimental diets Vitamins A, B_2 and B_6 were added to the nine experimental diets (T1 to T9) as shown in Table 1. The main nutritional components of the basic experimental powder diets were measured (Table 2). To determine the nutrient digestibility, 0.1% chromium oxide, an inert marker, was added to each diet. The powder diets were blended with water (35%), formed into wet pellets and stored at –20°C.

2.3 Experimental animals and procedures The turtles were acclimated to the laboratory conditions for 3 weeks in 135 aquaria [60 cm (l) × 30 cm (w) × 30 cm (h), water volume of 20 L] and fed the T1 diet. The

Table 1 The orthogonal experimental design for analysis of the vitamins.

Treatment	VA (IU kg^{-1})	VB_2 (mg kg^{-1})	VB_6 (mg kg^{-1})
T1	5000	60	20
T2	5000	120	70
T3	5000	180	120
T4	20,000	60	70
T5	20,000	120	120
T6	20,000	180	20
T7	35,000	60	120
T8	35,000	120	20
T9	35,000	180	70

Table 2 The ingredients and nutrient composition of the experimental diets.

	Ratio/ Content (%)
Ingredient [a]	
White fish meal	53
Squid liver meal	5
Yeast meal	5
Expanded soybean meal	8
α-starch	18
Fish oil	2.2
Whey meal	1
Calcium hydrogen phosphate	2.6
Calcium carbonate	1.5
Potassium chloride	0.2
Sodium sulfate	0.2
Salt	0.35
Zeolite	0.25
Methionine	0.1
Lysine	0.27
Betaine	0.2
Choline chloride	0.1
Preservatives	0.03
Vitamin premix [b]	1.2
Mineral premix [c]	0.8
Composition	
Moisture	6.09
Crude protein	40.28
Crude lipid	7.63
Carbohydrate [d]	33.09
Crude ash	12.91
Energy (KJ/g)	16.67

[a] Ingredients of the experimental diets were based on the natural dry mass.
[b] Vitamin premix (IU or mg/kg of diet): D, 5,000 IU/kg; E, 350 mg/kg; K, 50 mg/kg; B_1, 70 mg/kg; B_{12}, 1 mg/kg; Ca pantothenate, 320 mg/kg; nicotinic acid, 400 mg/kg; folic acid, 20 mg/kg; inositol, 500 mg/kg; C, 700 mg/kg; biotin, 1 mg/kg; choline chloride, 1,000 mg/kg (the contents of VA, VB_2 and VB_6 were listed in Table 2).
[c] Mineral premix (mg/kg of diet): $MnSO_45H_2O$, 200 mg/kg; $CuSO_45H_2O$, 20 mg/kg; $ZnSO_47H_2O$, 260 mg/kg; $FeSO_47H_2O$, 300 mg/kg; KI, 0.50 mg/kg; CoCl, 0.10 mg/kg; $NaSeO_3$, 0.3 mg/kg.
[d] Carbohydrate content was calculated as the remainder of diet (dry matter) after subtracting crude protein, crude lipid, and crude ash.

water temperature was maintained at 30±0.5°C using a thermostat-controlled electric heater. The photoperiod was maintained at 14L:10D, with illumination between 07:00 and 21:00. The pH ranged from 7.5 to 8.0, and the DO content was over 6 mg L^{-1}.

We randomly allocated 135 turtles to the aquaria, with one turtle per aquarium. The average body weight of the turtles was 5.90±0.20 g (weight±SD). The turtles were fed their respective diets at a rate of 4% body weight per day twice daily at 08:00 and 16:00. Uneaten feed was collected, and feces were removed after 30 minutes of feeding and were then dried at 60°C to a constant weight. Approximately one-third of the water in each aquarium was exchanged every day to maintain the water quality. The experiment continued for 80 days.

2.4 Sample collection and measurement Prior to the experiment, 15 turtles were randomly collected for collecting the initial samples. At the end of the experiment, all turtles from each group were sampled. The protein contents of all turtle samples were measured. The diets, uneaten feed, feces and turtles were dried at 60°C to a constant weight and were then smashed and sieved using a sample sifter. The crude protein contents of the samples were determined using the Kjeldahl method, and their energy contents were measured using a calorimeter (DJL-9, Changsha Xingdian Instrument, Changsha, Hunan, China).

2.5 Data calculation The survival rate(SR), feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), apparent digestibility coefficient of dry matter (ADC), protein digestibility coefficient (PDC), protein efficiency rate (PER), protein deposition rate (PDR) and energy efficiency (EGE) were calculated as follows:

$SR (\%) = 100 \times N_2 / N_1$

$FI (\%) = 100 \times F / [T (W1 + W2) / 2]$,

$SGR (\%d^{-1}) = 100 (\ln W_2 - \ln W_1) / T$

$FCR = F / (W_2 - W_1)$

$ADC (\%) = 1 - [(Cr_2O_3 \text{ in diet} / Cr_2O_3 \text{ in feces}) \times 100\%$

$PDC (\%) = 1 - [(Cr_2O_3 \text{ in diet} / Cr_2O_3 \text{ in feces}) \times (\text{protein in feces} / \text{protein in diet})] \times 100\%$

$PER (\%) = 100 (W_2 - W_1) / F_p$

$PDR (\%) = 100 \times B_p / F_p$

$EGE (\%) = 100 \times G / (C–F)$

where N_1 and N_2 are the initial and final numbers of turtles in each tank, respectively; W_1 and W_2 are the initial and final body weights of the turtles (g), respectively; T is the duration of the experiment (d); F is the cumulative feed intake; F_p is the protein intake; and B_p is body protein gain.

G, C and F (kJ) are growth energy, intake energy, and faecal energy, respectively, in the energy budget equation (C = G + F + U + R); and C–F represent the energy assimilated by the turtles.

2.6 Data calculation and statistical analyses The importance of the three vitamins for growth was evaluated based on the effectiveness of each vitamin according to calculated ranges (R) (Roy 1990) and the difference between the mean maximum and minimum values of each index at the three vitamin levels, which indicated the most influential factor (i.e., the factor resulting in the greatest improvement) for growth performance (Yan *et al.*, 2009).

The data were analyzed using Statistica 6.0 software (Statsoft Inc., Tulsa, OK, USA). One-way ANOVA was used to detect the differences among the treatment means at a 5% significance level, and Duncan's multiple range test was used to evaluate the differences among the treatment means.

3. Results

3.1 Survival rate, feed intake and growth There was no mortality during the 80 days of this experiment. The results revealed that the feed intake was the highest for the T3 diet, with significantly higher intake than the T5 or T6 diet (F = 1.46, df = 134, $P_{3,5}$ = 0.049, $P_{3,6}$ = 0.040) (Table 3). The feed intake ranges (R) for the three vitamins at the three levels varied from 0.038 to 0.083, and VA exhibited the largest range (Table 4). The order of importance of the vitamins to feed intake was VA>VB_2>VB_6, and the vitamin combination and levels resulting in the highest feed intake was A_3, B_{23}, and B_{63} (Table 4).

There were no significant differences in the SGR among the treatments (F = 0.822, df = 134, P = 0.58). The SGR ranges (R) for the 3 vitamins varied from 4.8% to 18.4%, and VA exhibited the largest range. The order of importance of the vitamins to the SGR was VA>VB_2>VB_6, and the optimal vitamin combination for achieving the highest SGR was A_3, B_{23}, and B_{62} (Table 4).

3.2 Dietary nutrient utilization The FCR, PER, PDR, ADC and PDC are listed in Table 5. There were no significant differences in the ADC or PDC among the groups analyzed (F_{ADC} = 0.63, df = 134, P_{ADC} = 0.72; F_{PDC} = 0.85, df = 134, P_{PDC} = 0.92). The ADC ranges (R) varied from 0.25 to 1.25, and VA exhibited the largest range. The order of importance of the 3 vitamins to the ADC and PDC was VA>VB_2>VB_6, and the optimal vitamin combinations were A_2, B_{22}, and B_{63} for the ADC and A_2, B_{21}, and B_{63} for the PDC (Table 6).

During the experiment, no significant differences in the PER or FCR were detected among the nine treatment groups (F_{PER} = 0.67, df = 134, P_{PER} = 0.558; F_{FCR} = 0.64, df = 134, P_{FCR} = 0.74). The order of importance of the 3 vitamins to the PER and FCR was VA>VB_6>VB_2, and the optimal vitamin combination was A_2, B_{22}, and B_{61} of vitamins for the PER and FCR (Tables 6 and Table 7).

T6 yielded a higher PDR than T1, T3 and T9 (F = 1.32, df = 134, $P_{6,1}$ = 0.049, $P_{6,3}$ = 0.035, $P_{6,9}$ = 0.043). The order of importance of the 3 vitamins to the PDR was VB_2>VA>VB_6, and the optimal vitamin combination was A_2, B_{22}, and B_{61} for the PDR (Table 7).

3.3 Energy utilization There were significant differences in the energy intake among the nine groups (F_{EI} = 1.06, df = 134, P_{EI} = 0.041) (Table 8). Group T3 exhibited the greatest energy intake, which was significantly higher than those of groups T2, T5, and T6 ($P_{3,2}$ = 0.04, $P_{3,5}$ = 0.04, $P_{3,6}$ = 0.032). The energy intake ranges (R) for the three vitamins varied from 6.1 to 12.69, and VA exhibited the largest range. The order of importance of the vitamins with regard to energy intake was VA>VB_2>VB_6, and the vitamin combination resulting in the greatest energy intake was A_1, B_{23}, and B_{63} (Table 9).

Significant differences in the energy efficiency for growth were also observed (F_{EGE} = 1.06, df = 134, P_{EGE} = 0.041); that of group T6 produced was greater than those of groups T1, T3, T4, T7, T8, and T9. The order of importance of the vitamins with regard to the energy efficiency for growth was VB_2>VA>VB_6, and the vitamin combination resulting in the greatest energy efficiency for growth was A_2, B_{22}, and B_{61} (Table 9).

4. Discussion

Assessment of appropriate vitamin combinations may provide a more realistic representation of the vitamin requirements of animals, as appropriate combinations of VB_2, VB_6, niacin and pantothenic acid have been shown to improve the growth and meat quality of crucian carps (Lin *et al.*, 2003). In the present study, no mortality, avitaminosis or hypervitaminosis was observed during the experiment, and the results indicated that dietary supplementation with the different combinations of VA, VB_2 and VB_6 did not significantly affect the SR of the soft-shelled turtles. The results also demonstrated that the vitamin combinations clearly affected the FI, PDR and EGE of the reptiles (P<0.05).

In the present study, VA had much greater effects on the FI, SGR, ADC, PDC, FC and PER than VB_2 and VB_6 (Tables 4, 6 and 7), indicating that VA plays

Table 3 Effects of the different diets on feeding and growth of *Pelodiscus sinensis*.

Treatment	Initial weight (g ind^{-1})	Final weight (g)	Feed intake (%)	Specific growth rate (%d^{-1})
T1	5.908±1.041	59.08±19.83	$1.836±0.120^{ab}$	2.814±0.502
T2	5.906±0.933	63.13±29.04	$1.756±0.161^{b}$	2.813±0.591
T3	5.919±0.987	58.25±21.55	$1.903±0.253^{a}$	2.767±0.510
T4	5.905±0.963	54.21±21.73	$1.797±0.082^{ab}$	2.697±0.353
T5	5.914±0.742	53.21±23.22	$1.749±0.175^{b}$	2.651±0.439
T6	5.913±1.185	59.34±21.13	$1.721±0.165^{c}$	2.829±0.534
T7	5.912±1.206	63.60±21.66	$1.828±0.133^{ab}$	2.934±0.483
T8	5.907±0.973	60.52±26.27	$1.811±0.263^{ab}$	2.808±0.491
T9	5.908±1.005	66.52±22.47	$1.876±0.074^{ab}$	2.985±0.472

Note: The values are presented as the mean ± SD (n=15); the different letters indicate a significant difference between the treatments (P<0.05).

Table 4 Results of analysis of the effects of different vitamin levels on feed intake and growth.

Item	Feed intake (%)			Specific growth rate (%d^{-1})		
	A	B_2	B_6	A	B_2	B_6
K1	1.832	1.821	1.789	2.798	2.815	2.817
K2	1.756	1.772	1.81	2.725	2.757	2.832
K3	1.839	1.834	1.827	2.909	2.86	2.784
Optimal level	3	3	3	3	3	2
R	0.083	0.062	0.038	0.184	0.103	0.048
Order of importance		$A>B_2>B_6$			$A>B_2>B_6$	

K1, K2 and K3 represent the feed intake rates and specific growth rate means at levels 1, 2 and 3, respectively. Optimal level indicates the level that yielded the best feed intake rate or specific growth rate. R (range) represents the difference between the maximum and minimum average feed intake and the specific growth rates. Order of importance indicates the order of importance or effectiveness of the 3 vitamins with regard to the index.

Table 5 Effects of the different diets on diet utilization in *Pelodiscus sinensis*.

Treatment	Apparent digestibility coefficient (%)	Protein digestibility coefficient (%)	Protein deposition rate (%)	Protein efficiency rate (%)	Feed conversion ratio
T1	85.80±1.20	93.26±0.49	$44.44±3.43^{b}$	270.6±31.6	0.9296±0.1102
T2	86.25±0.19	93.38±0.91	$46.87±4.37^{ab}$	282.1±36.9	0.8950±0.1211
T3	85.85±0.48	93.22±0.20	$43.80±5.91^{b}$	262.2±46.6	0.9822±0.2103
T4	87.48±0.16	94.23±0.16	$44.92±2.48^{ab}$	272.3±21.8	0.9176±0.0812
T5	87.36±0.55	94.12±0.23	$47.27±4.77^{ab}$	278.1±35.1	0.9067±0.1236
T6	86.87±0.29	94.09±0.23	$48.13±4.04^{a}$	289.8±29.4	0.8659±0.0910
T7	86.64±0.14	94.10±0.17	$46.14±3.22^{ab}$	277.6±24.9	0.8922±0.1025
T8	86.90±0.80	93.69±0.45	$45.59±6.38^{ab}$	279.1±46.5	0.9225±0.2219
T9	85.37±0.71	93.01±0.34	$44.01±2.55^{b}$	275.5±25.6	0.9079±0.0934

Note: The values are presented as the mean ± SD (n=15); the different letters indicate a significant difference between the treatments (P<0.05).

Table 6 Results of analysis of the effects of different vitamin levels on diet utilization.

Item	Apparent digestibility coefficient (%)			Protein digestibility coefficient (%)			Feed conversion ratio		
	A	B_2	B_6	A	B_2	B_6	A	B_2	B_6
K1	85.98	86.65	86.54	93.29	93.86	93.68	0.9356	0.9131	0.906
K2	87.23	86.84	86.37	94.15	93.73	93.54	0.8967	0.9081	0.9068
K3	86.31	86.03	86.62	93.6	93.44	93.82	0.9075	0.9186	0.927
Optimal level	2	2	3	2	1	3	2	2	1
R	1.25	0.81	0.25	0.86	0.42	0.27	0.0389	0.0106	0.021
Order of importance		$A>B_2>B_6$			$A>B_2>B_6$			$A>B_6>B_2$	

K1, K2 and K3 represent the protein digestibility at levels 1, 2 and 3, respectively. Optimal level indicates the vitamin level that resulted in the best ADC, PDC, and FCR values.R (range) indicates the difference between the maximum and minimum average of the index. Order of importance indicates the order of importance or effectiveness of the 3 vitamins with regard to the index.

Table 7 Results of analysis of the effects of different vitamin levels on PDR and PER.

Item	Protein deposition rate			Protein efficiency rate		
	A	B_2	B_6	A	B_2	B_6
K1	45.4	45.43	46.29	271.7	273.5	279.9
K2	46.39	46.71	46.09	280.1	279.8	276.7
K3	46.02	45.67	45.42	277.4	275.9	272.7
Optimal level	2	2	1	2	2	1
R	0.99	1.28	0.87	8.4	6.3	7.2
Order of importance		B_2>A>B_6			A>B_6>B_2	

K1, K2 and K3 represent the protein digestibility at levels 1, 2 and 3, respectively. Optimal level indicates the vitamin level that yielded the best protein deposition and protein efficiency rates. R (range) indicates the difference between the maximum and minimum average of the index. Order of importance indicates the order of importance or effectiveness of the 3 vitamins with regard to the index.

Table 8 Effects of the different diets on energy intake and net energy efficiency for growth.

Treatment	Energy intake (kJ)	Energy efficiency (%)
T1	306.08 ± 20.11^{ab}	35.51 ± 3.75^{bc}
T2	292.70 ± 27.13^{b}	37.92 ± 4.35^{ab}
T3	317.33 ± 42.23^{a}	34.66 ± 5.71^{c}
T4	299.63 ± 13.83^{ab}	35.15 ± 2.60^{bc}
T5	291.58 ± 29.37^{b}	38.30 ± 4.46^{ab}
T6	286.84 ± 26.83^{b}	39.88 ± 3.86^{a}
T7	304.27 ± 21.84^{ab}	36.19 ± 2.96^{bc}
T8	301.95 ± 44.21^{ab}	36.08 ± 5.69^{bc}
T9	309.16 ± 12.87^{ab}	36.37 ± 2.99^{bc}

Note: The values are presented as the mean ± SD (n=15); the different letters indicate a significant difference between the treatments (P<0.05).

Table 9 Results of analysis of the effects of different vitamin levels on energy intake and energy efficiency for growth.

Item	Energy intake (kJ)			Energy efficiency (%)		
	A	B_2	B_6	A	B_2	B_6
K1	305.37	303.33	298.29	36.03	35.62	37.16
K2	292.68	295.41	300.5	37.78	37.43	36.48
K3	305.13	304.44	304.39	36.22	36.97	36.39
Optimal levels	1	3	3	2	2	1
R	12.69	9.03	6.1	1.75	1.82	0.77
Order of importance		A>B_2>B_6			B_2>A>B_6	

K1, K2 and K3 represent the energy intake or energy efficiency for growth at levels 1, 2 and 3, respectively. Optimal level indicates the level that yielded the greatest energy intake or energy efficiency for growth. R (range) indicates the difference between the maximum and minimum average energy intake or energy efficiency for growth. Order of importance indicates the order of importance or effectiveness of the 3 vitamins with regard to the index.

important roles in multiple processes, including those related to digestion, nutrient utilization and growth. VA supplementation at level 2 improved digestive functions (ADC, PDC, FC, PDR and PER) more than that at level 1 or 3. Further, VA supplementation at level 3 had greater effects on the SGR than that at the other levels, indicating that a high VA level (35,000 IU kg^{-1}) can improve the feeding and growth rate of the soft-shelled turtles. The above results demonstrate that VA plays a broad and important role in juvenile turtle growth. Previous studies have suggested that the VA requirements of most finfish range from 1000 to 20,000 IU kg^{-1} (Masumoto, 2002; Mohamed *et al*., 2003; Moren *et al*., 2004; Hernandez *et al*., 2005). Based on the appropriate levels, dietary VA supplementation at 20,000-35,000 IU kg^{-1} should be used for soft-shelled turtles. The differing demands for VA between these two animals may be attributed to differences in metabolic processes (Chen and Huang, 2014). In contrast with the present study, the recommended dietary VA requirement for turtles was found to be 10800-11600 IU kg^{-1} in the aforementioned study (Chen and Huang, 2014), and the turtle growth (WG, FCR and PER) in the present study was superior to that in this previous study. The discrepant results between

two studies may be due to the use of different ingredients, nutrient compositions and VA supplementation levels in the diets. In the present study, VA supplementation at level 3 (35,000 IU kg^{-1}) had more beneficial effects on the turtle growth rate than that at level 2 (20,000 IU kg^{-1}).

In this study, VB_2 had greater influences on the PDR and EGE than VA and VB_6 based on the relative orders of importance of these vitamins, which is consistent with the finding that the whole-body protein content in Jian carp increases with an increasing dietary riboflavin levels (Li *et al*., 2009). The results of this study indicate that VB_2 may play an important role in converting dietary protein and energy into usable protein and energy in the soft-shelled turtle. In previous studies, VB_2 at a suitable level has been shown to be conductive to the growth of some aquaculture animals (Xu *et al*., 1995; Souto *et al*., 2008; Li *et al*., 2010). Souto *et al*. (2008) have found that sea bream fed a VB_2- enriched diet (17.7 mg kg^{-1}) grew better than those fed a control diet (13.7mg kg^{-1}). In addition, a low dietary VB_2 level (100 mg/kg) has been shown to result in a higher SGR than a high dietary VB_2 level (400mg kg^{-1}) in shrimp (Xu *et al*., 1995), perhaps due to the high levels of digestive enzymes and energy necessary for separating VB_2 from proteins (Wang and Shan, 2007). In the present study, VB_2 supplementation at level 2 (120 mg kg^{-1}) resulted in the optimal rates of absorption and conversion of protein and energy (Tables 7 and 9), and that at level 3 (180 mg kg^{-1}) yielded an optimal growth rate compared with that at the other two levels; thus, the VB_2 level in the juvenile turtle diet should be approximately 120–180 mg kg^{-1}.

Previous experiments have demonstrated that VB_6 influences the PER and feed coefficient ratio (FCR). The metabolism of this vitamin is related to dietary protein or amino acid metabolism in animals (Hilton, 1989; Giri *et al*., 1997). In the present study, VB_6 had fewer effects on protein metabolism than VA based on the order of importance of the vitamins (Tables 6 and 7). Further, VB_6 had a greater influence on the FCR than VB_2, and the same result has been found in a study conducted by Lin *et al*. (2003) showing that VB_6 has important effects on digestive enzyme and alkaline phosphatase activities (He *et al*., 2009). The bass *Lateolabrax japonicus* and Jian carp *Cyprinus carpio* exhibit optimal growth atVB_6 concentratons of 20 mg kg^{-1} (Zhong and Zhang, 2001) and 6.07mg kg^{-1} (He *et al*., 2009), respectively. Further, the most appropriate VB_6 level for shrimp is approximately 140 mg kg^{-1} (Xu *et al*., 1995). In the present study, based on the PER and FCR K values, VB_6 supplementation at level 1 (20 mg kg^{-1}) was optimal compared with that at the other levels, and the PER and FCR gradually worsened with increasing VB_6 levels (Tables 6 and 7). In addition, VB_6 supplementation at level 2 (70 mg kg^{-1}) resulted in a higher SGR of the turtles (Table 4). Therefore, VB_6 should be kept at a low level (20–70 mg kg^{-1}) in the juvenile turtle diet.

The results of this study demonstrated that the order of importance of the 3 vitamins with regard to the turtle feed intake, growth and digestibility was VA>VB_2>VB_6 and that the order of importance with regard to the conversion capacity was VB_2>VA>VB_6 (Tables 4, 6, and 7). These findings suggest that at the levels tested, VA influenced feeding, growth, digestion and feed utilization, and had the strongest effects on the soft-shelled turtles, that VB_2 played an important role in growth efficiency (PDR and EGE), and that VB_6 had greater effects on the FCR and PER than did VB_2.

The results showed that the vitamin combination A_2, B_{22}, and B_{61} generated the highest PDR and PER and that combination A_3, B_{23}, and B_{62} resulted in optimal growth; thus, based on the growth results, the dietary VA, VB_2 and VB_6 requirements for soft-shelled turtles were estimated to be 35,000 IU kg^{-1}, 180 mg kg^{-1} and 70 mg kg^{-1}, respectively.

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